

17
Bioavail ability
Particle size

Effect of Particle Size on Lead Absorption from the Gut

D. BARLTROP, M.D., F.R.C.P.
F. MEEK, A.A.I.M.T., A.R.M.I.T.
Department of Child Health
Westminster Children's Hospital
Vincent Square
London, England

ABSTRACT. The relationship between particle size and absorption of lead particles from the gastrointestinal tract of the rat has been investigated. Preparations of metallic lead of particle size between 0 and 250μ were incorporated in laboratory rat diets and absorption determined by measurement of tissue lead concentrations attained under standard conditions. An inverse relationship was found between particle size and lead absorption; this relationship was most marked in the 0 to 100μ range. A five-fold enhancement of absorption was observed from the diet with lead particles of mean size 6μ , compared with 197μ particle size.

Lead absorption from dried paint films containing lead chromate and lead octoate was measured using a similar technique. A marked enhancement of absorption was observed for both paints when particle size was reduced from 500 to $1,000\mu$ to $< 50\mu$.

PARTICLE SIZE has long been recognized to influence the retention of atmospheric lead (Pb) in the respiratory tract, but there is little corresponding information concerning the gut. The two situations are not entirely analogous since all ingested Pb particles are potentially available for absorption, whereas only the small particle fraction of atmospheric Pb is likely to reach an absorptive surface in

the lung, and only part of this is retained and absorbed.

Lead-containing soils and dusts are being seen as a potential hazard, especially for children. Contaminated dusts have been suspected as potential sources of poisoning in relation to Pb smelters.^{1,2} Much of the continuing concern with regard to Pb additives in gasoline has now become focused on the ultimate deposition of Pb from automobile exhausts on soils and dusts.

Evidence has been presented which suggests that about one-third of atmospheric Pb derived from automobile exhausts might be retained in the respiratory tract. The remaining fraction is either exhaled, or in the case of large particles, deposited in the major airways where they are entrapped in mucus secretions, transported by ciliary action to the larynx, and ultimately swallowed. It has been presumed that swallowed particles of atmospheric origin will result in less absorption than those in the inhaled fraction, since it is generally accepted that only 10% of dietary lead is absorbed from the gut.³

Children exposed to heavily contaminated soils and dusts are known to sustain an increased soft tissue Pb burden, but few observations have been made in which the magnitude of this contribution has been determined. One study in a rural area of England, which was free from atmospheric pollution, showed that the mean blood lead concentration in children was 8 to $9\mu\text{g}/100\text{g}$ greater in a district where the mean soil-lead content was 13,900 ppm

compared with controls living in an area with 4,000 ppm mean soil lead content.⁴ In that study no relationship was found with pica for soil, but it was recognized that both the physical and chemical forms of Pb might have modified its availability for absorption.

This paper reports studies in the rat designed to determine the influence of particle size on the availability of Pb for absorption from the gut. The experimental model has already been described.⁵ The studies were conducted with both metallic Pb and with material derived from two dried paint films containing lead chromate and lead octoate, respectively.

Methods

In previous studies a standardized procedure for the determination of Pb absorption was established using 30- to 32-day-old male Wistar rats weighing 90 to 110 g.⁵ These experimental conditions were chosen to minimize variation in Pb absorption which might be associated with animal age and maturity. In the first study using metallic Pb 30-day-old male Wistar rats of mean body weight 93 g were used. Lead was added to the diets to a concentration of 0.075%. Two rats were killed prior to the trial and blood and kidneys analyzed for Pb to ensure that the animals had not previously been exposed to Pb. Thirty-six rats were allocated at random into six groups of six animals, with each rat housed individually in a stainless steel and polythene cage. Tap water containing less than 10 µg/l Pb was supplied ad libitum. Diets were prepared from Oxoid 41B (modified) powdered diet and made into stick form using 5% molasses. The method of diet preparation resulted in a uniform distribution of Pb and homogeneity was confirmed by analysis of sequential portions.

Metallic Pb (Lead Powder, B.D.H.) was sieved into six particle size ranges using Endacott's Scientific Test Sieves and each of these was used to prepare the diets for one group of animals.

Particle size distribution for each portion was verified by means of a double image micrometer and the mean particle size, range, and mean surface area calculated (Table 1). Examination of the particles by conventional microscopy showed that they were irregular in shape (Fig. 1) so that the particle surface areas which were calculated on the assumption of spherical form are approximate. Each animal was provided with a weighed portion (31 to 33 g) of the appropriate diet for the 48-hr test period.

Table 1.—Metallic Lead Particle Size

Group	Mean (µ) ± 2 SD	Mean Surface Area (µ ²)
1	6 ± 9	128
2	49 ± 19	7,529
3	79 ± 56	19,456
4	107 ± 70	35,832
5	137 ± 118	59,153
6	197 ± 166	121,715



Fig. 1. Micrograph of metallic lead particles in the 30 to 68µ range.

The second study was designed to determine the absorption of Pb from powdered films differing in particle size. Two paints were used which contained 0.48% Pb as lead octoate and 0.39% Pb as lead chromate, respectively. The corresponding diets were prepared to contain 0.02% Pb in order to restrict the paint content of the diet to a palatable level. A lead acetate diet was also prepared to this concentration to act as a standard for comparison. Each paint was incorporated into the rat diets in two particle size ranges: 500 to 1,000 µ and < 53 µ; the lead acetate was also < 53 µ in particle size. These particle sizes were determined using Endacott's Scientific Test Sieves. Six groups of 31-day-old male Wistar rats, 90 to 103 g body weight, were allocated randomly in separate cages and supplied with a weighed portion (31 to 33 g) of the prepared diets and tap water ad libitum (Table 2).

The rats were killed 48 hr after the initiation of Pb feeding and dissected immediately. Blood was obtained by venepuncture and collected into heparinized lead-free tubes. The kidneys were removed, sealed in polythene bags and frozen at -20°C pending analysis. Any remaining food was weighed and the Pb intake determined by difference. Lead analysis of blood, kidneys, and diets were made after wet-ashing with a sulphuric, nitric, perchloric acid digestion mixture, by a semi-automated dithizone technique.⁶

Table 2.—Diets for Paint Film Study

Animal Group	Diet Supplied
1	Control
2	Lead acetate (0.02% Pb)
3	Lead octoate paint 500-1000µ particle size
4	Lead octoate paint < 50µ particle size
5	Lead chromate paint 500-1000µ particle size
6	Lead chromate paint < 50µ particle size

Table 3.—Metallic Lead Study: Blood and Kidney Lead Content*

Animals Group†	Blood Lead ($\mu\text{g}/100\text{g}$)	Total Kidney Lead (μg)
1	30.5 ± 4.7	7.13 ± 0.82
2	23.0 ± 1.8	3.75 ± 0.58
3	18.3 ± 5.0	2.28 ± 0.70
4	19.1 ± 3.3	2.13 ± 0.58
5	14.5 ± 2.1	1.35 ± 0.48
6	13.5 ± 1.6	1.15 ± 0.21
Controls	8.3 ± 1.9	0.92 ± 0.25

*Mean \pm SD.

†Groups 1 to 6 fed diets containing 0.075% lead.

Results

Metallic Lead

The rats were 32-days old and weighed 101 to 119 g when sacrificed. The tissue lead content for blood and kidney in each group is given in Table 3. A marked inverse relationship between tissue lead content and the particle size of the ingested Pb was observed. Thus, the mean blood lead concentrations for animals fed preparations of Pb < 38 μ diameter was 2.3 times greater than that observed in animals fed particles of 150 to 250 μ diameter. Similarly, the mean kidney lead content for animals receiving the small particle size preparation was 6.2 times that of animals fed with preparations of large particle size. Plotting

the tissue content data against particle size gave exponential curves which were similar for blood and kidney (Figs. 2 and 3).

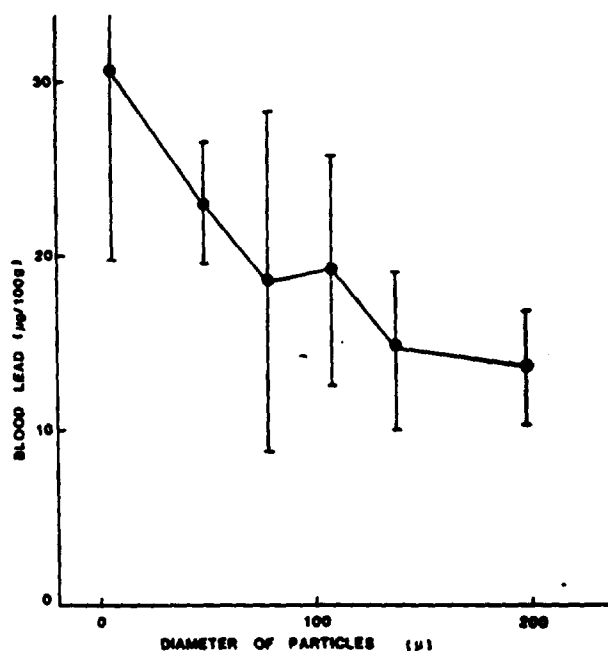
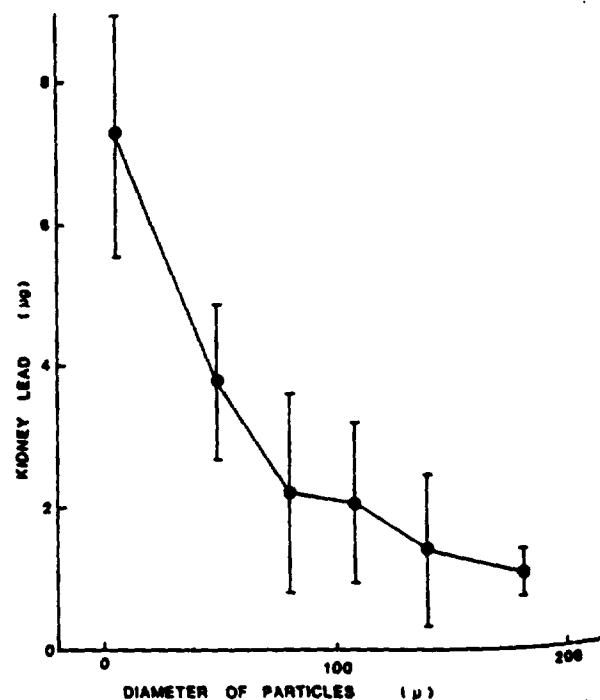
One interpretation of the observed relationship might be that the relative proportion of lead oxides to metallic Pb (due to surface oxidation) might be increased in the particles of small diameter. Lead oxide has been shown to be well absorbed from the intestinal tract of the rat.⁵ Each of the various particle size preparations of Pb was, therefore, examined by means of x-ray diffraction. It was found that the proportion of lead oxide to lead increased from a trace for particles of mean diameter 197 μ to approximately 5% for particles of mean diameter 6 μ .

Blood lead concentration and kidney lead content were found to be related to the surface area of the ingested particles. Plotting tissue lead data against surface area gave curves which were essentially similar to those obtained for plots against particle diameter, but with a more marked inflexion of the curve at surface areas of approximately 29,000 μ^2 (Figs. 4 and 5).

Paint Study

The results of blood lead concentrations and kidney lead contents are given in Table 4. The same absorption ratio was observed between the particles from paint films containing lead octoate and the film containing lead chromate as had been demonstrated between two pure compounds in previous experiments.⁵ In each case the smaller-sized particles were associated with an increased tissue lead content which was more marked for kidney than for blood (Figs. 6 and 7).

Decreasing particle size of the paint films from 500 to 1,000 μ to less than 50 μ resulted in a 1.5- to 1.6-fold

Fig. 2. Effect of metallic lead particle size on blood lead concentration ($N = 6$).Fig. 3. Effect of metallic lead particle size on total kidney lead ($N = 6$).

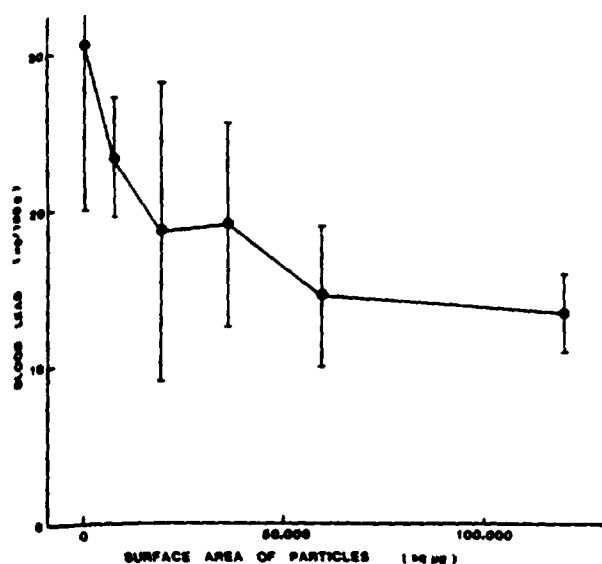


Fig. 4. Effect of surface area of metallic lead particles on blood lead ($N = 6$).

increase in blood lead concentration and a 1.5- to 1.8-fold increase in kidney lead content. In neither case did the tissue lead concentrations achieved attain those of the animals fed lead acetate. The maximal tissue lead contents in the paint film studies were found in association with the lead octoate films of $< 50 \mu$ particle size. In this case tissue lead concentrations which were 71% and 67% of those for animals fed lead acetate were found for blood and kidney lead, respectively.

Comment

The data for metallic Pb confirm that there is a marked relationship between particle size and availability for absorption from the gut. This relationship was such that a five-fold enhancement of absorption was observed in the range from 6μ to 197μ mean diameter of particles. The slope of the curve obtained suggested that greater absorp-

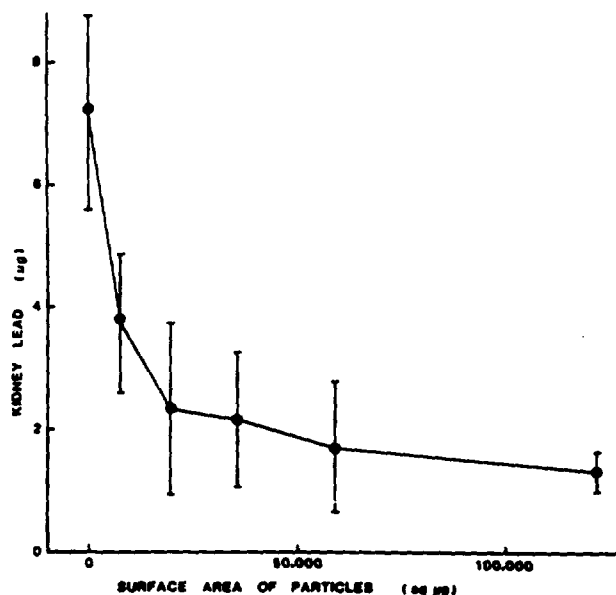


Fig. 5. Effect of surface area of metallic lead particles on total kidney lead ($N = 6$).

tion would be expected for smaller particles in the range of 0 to 6μ which might be encountered in retained and swallowed material from atmospheric sources. The findings indicate, therefore, that it may not be possible to derive a single retention factor for dietary Pb and it follows that the potential hazard associated with ingested material derived in various environmental situations may differ.

Some caution is required before these data are extrapolated to human populations. It has been shown that the rat only absorbs 1% of ingested Pb even from the more readily absorbed compounds such as lead acetate and lead oxide, contrasting with 10% dietary retention usually attributed to the human adult.³ This may reflect species differences in diet rather than a difference in absorptive capacity. Modification of the diet in the rat has been shown to result in marked differences in the degree to which a given compound of Pb may be absorbed, in that a low-mineral, high-fat diet will enhance the absorption of Pb up to 50 times control values.⁷

Table 4.—Paint Film Study: Blood and Kidney Lead Content*

Lead Compound	Particle Size (μ)	Blood Lead $\mu\text{g}/100\text{g}$	Total Kidney Lead μg
None (control)	—	8.1 ± 1.9	0.51 ± 0.07
Lead acetate	< 50	38.3 ± 4.0	6.45 ± 1.16
Lead octoate (paint)	500-1000	19.3 ± 3.7	2.38 ± 0.29
Lead octoate (paint)	< 50	27.2 ± 4.0	4.30 ± 0.46
Lead chromate (paint)	500-1000	14.5 ± 3.2	2.18 ± 0.67
Lead chromate (paint)	< 50	22.8 ± 2.2	3.35 ± 0.46

*Mean \pm SD.

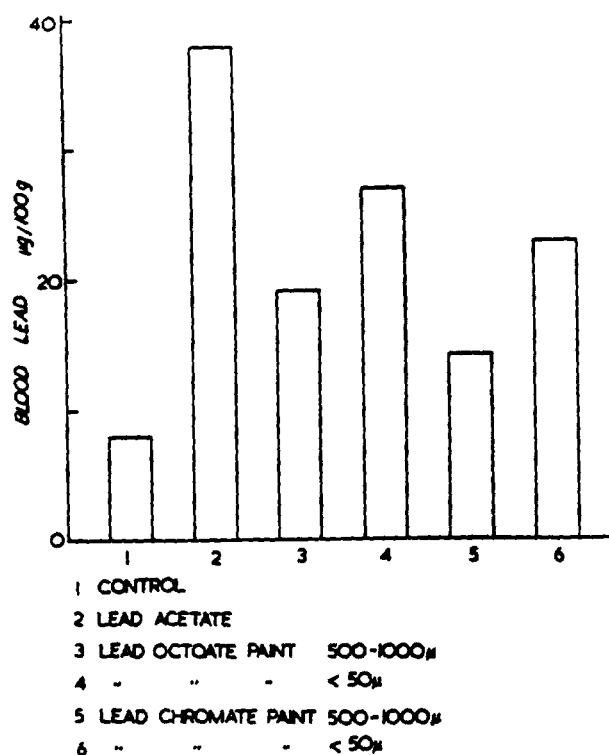


Fig. 6. Relative blood lead concentrations for different particle size paint films fed to rats ($N = 6$).

A possible explanation for the enhanced absorption of Pb from small particles might be the increase in surface oxidation forming products which are more readily absorbed.⁸ However, x-ray diffraction studies showed that the smallest particle size range contained only approximately 5% lead oxides which would be insufficient to account for the observed increases in the Pb content of blood and kidney.

It is recognized that particle size measurements reported in this paper refer to Pb added to the diet and do not necessarily reflect the state of the added Pb in the prepared diet or in the gut lumen. It is possible that agglomeration of particles may have occurred during diet preparation or after ingestion in the gut. The effect of this would have been to increase the mean particle size of the added Pb and thus to impair absorption. For example, particles not incorporated into diets—large particles which are inhaled and trapped in large air passages and subsequently swallowed—may have a greater absorption.

The physical form of particles derived from paint film would seem to modify the availability of Pb compounds contained in them for absorption. Little is known of the physical or chemical changes which paint flakes undergo after ingestion, although it is known that some paint flakes remain relatively intact when swallowed by a child and may be observed radiographically in the gut lumen, or on inspection of feces. In spite of this, sufficient absorption of Pb resulting in childhood poisoning is known to occur, and in many cases the ingested flakes become too finely divided to be visible macroscopically. Thus the composi-

tion of the paint and the chemical nature of the added Pb compounds may determine its stability in the gut and hence the availability of Pb for absorption.

Long-term feeding of paint flakes identical to those used in this work, but of larger particle size (500 to 1,000 µ), have been reported to result in minimal absorption of Pb by the rat.⁹ This was attributed to the chemical nature of Pb additives which are relatively insoluble in the acid milieu of the stomach compared with basic lead carbonate used in older paint preparations. This view has been questioned in recent work in which the availability of Pb for absorption from various compounds has been measured.⁵

The present data suggest that particle size of the material derived from paint film is an important determinant of absorption. The potential hazard for children of Pb-containing surface-coating materials is determined by both the chemical form of the Pb additives and by the resistance to disintegration of the ingested flakes. It follows that it is unlikely that a single control measure can be devised which will be applicable to all paints or surface-coating materials and that it would be preferable to devise an alternative biological standard.

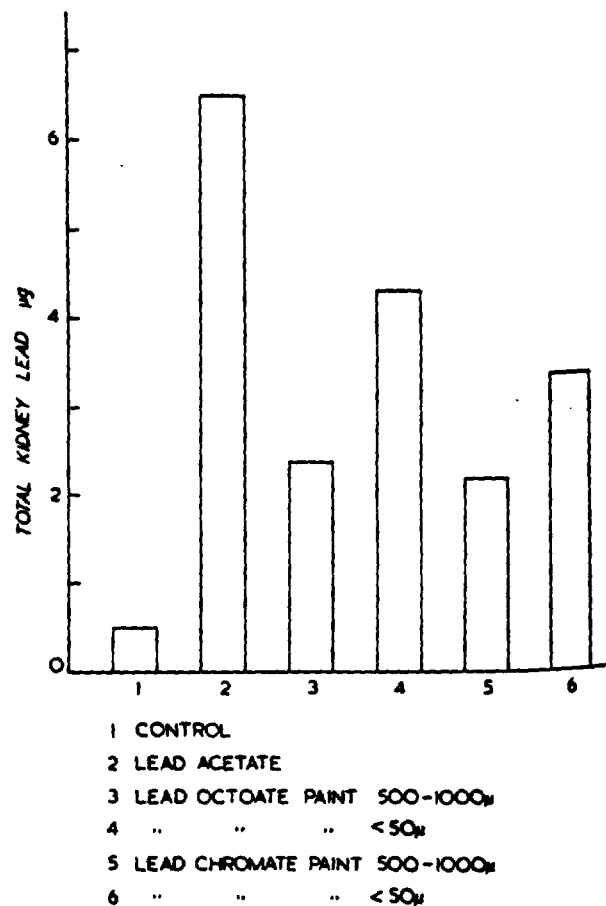


Fig. 7. Relative kidney lead content for different particle size paint films fed to rats ($N = 6$).

This work was performed pursuant to Contract No. HSM 99-73-28 of the Center for Disease Control, Department of Health, Education and Welfare, United States of America.

We thank Mr. D. M. Griffiths of the MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, for performing sizing analysis on the lead particle samples, and Mr. R. Curtis, Department of Geology, Imperial College, London, for determining the lead oxide content of these samples using x-ray diffraction techniques.

Submitted for publication September 9, 1976; revised; accepted for publication December 21, 1977.

REFERENCES

1. Lansdowne, R. G.; Shepherd, J.; Clayton, B. E.; Delves, H. T.; Graham, P. J.; and Turner, J. S. 1974. Blood lead levels, behaviour and intelligence. A population study. *Lancet* 1: 538-41.

2. McNeil, J. L.; Plasnik, J. A.; and Croft, D. B. 1975. Evaluation of longterm effects of elevated blood lead concentrations in asymptomatic children. *Proc. Int. Symp. on Environ. Lead Research, Dubrovnik, Arh Hig Rada Toksikol* 26: 97-118.
3. Kehoe, R. A. 1961. The Harben Lectures. The metabolism of lead in man in health and disease. *J Roy Inst Public Health* 24: 81-96, 101-120, 129-143, 177-203.
4. Barltrop, D.; Strehlow, C. D.; Thornton, I.; and Webb, J. S. 1974. The significance of high soil lead concentrations for childhood lead burdens. *Environ Health Perspect* 7: 75-82.
5. Barltrop, D. and Meek, F. 1975. Absorption of different lead compounds. *Postgrad Med J* 51: 805-09.
6. Browett, B. V. and Moss, R. 1965. Manual and semi-automated methods for the determination of the lead content of urine. *Analyst* London, 90: 715.
7. Barltrop, D. and Khoo, H. E. 1975. The influence of nutritional factors on lead absorption. *Postgrad Med J* 51: 795-800.
8. Barltrop, D. 1975. Assessment of the Health Hazard of Various Lead Compounds. Contract No. HSM 99-73-28. Report to D.H.E.W. (Available upon request from the Center for Disease Control, Atlanta, Georgia.)
9. Midwest Research Institute, Lead Paint Ingestion Study. 1974. Contract No. 62-W-62GC2NPC. (Available from the National Paint and Coatings Association Inc., Washington, D.C.)

Letter to the Editor

Betty R. Kuhnert, Ph.D.
Paul M. Kuhnert, Ph.D.
Perinatal Clinical Research
Center of Case Western
Reserve University
Department of Obstetrics
and Gynecology
Cleveland Metropolitan
General Hospital
3395 Scranton Road
Cleveland, Ohio 44109

To the Editor.—In their article "Plasma Cholinesterase Activity in Early Pregnancy," Howard et al.¹ reported a significant fall in plasma cholinesterase activity during the first three months of pregnancy, and presented preliminary evidence suggesting a return to normal pre-pregnancy levels later in pregnancy. In agreement with their results, previous investigators^{2,5} have also reported a decline in total enzyme activity during the first trimester but, in contrast, report that the serum levels remain fairly stable during the second and third trimesters. In our laboratory using the Kalow method,⁶ we have found the mean levels of plasma cholinesterase activity in laboring patients at term to be 45% lower than those in a control group of healthy nonpregnant women.⁷ It has been suggested that this decreased cholinesterase activity at term may be clinically important in women undergoing Cesarean deliveries who receive succinylcholine or other drugs metabolized by plasma cholinesterases.⁸

1. Howard, J.K.; East, N.J.; and Chaney, J.L., 1978. Plasma cholinesterase activity in early pregnancy. *Arch Environ Health* 33(5): 277.
2. Pritchard, J.A., 1955. Plasma cholinesterase activity in normal pregnancy and in eclamptogenic toxemias. *Amer J Obstet Gynecol* 70:1083.
3. Snider, S.M., 1965. Serum cholinesterase activity during pregnancy, labor and the puerperium. *Anesthesiology* 26(3):335.
4. Robertson, G.S., 1966. Serum cholinesterase deficiency II: Pregnancy. *Brit J Anaesth* 38:361.
5. Hazel, B., and Monier, D., 1971. Human serum cholinesterase: Variations during pregnancy and post-partum. *Canad Anaesth Soc J* 19(3): 272.
6. Kalow, W., and Genest, K., 1957. A method for the detection of atypical forms of human serum cholinesterase. Determination of Dibucaine numbers. *Canad J Biochem Physiol* 35:339.
7. Work in Progress.
8. Blitt, C.D.; Petty, W.C.; Albertarnst, E.E.; and Wright, B.J., 1977. Correlation of plasma cholinesterase activity and duration of action of succinylcholine during pregnancy, *Anesth Anal (Current Researches)* 56(1):78.